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Specific Activation of Puff 93D of *Drosophila melanogaster* by Benzamide and the Effect of Benzamide Treatment on the Heat Shock Induced Puffing Activity

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Abstract. A 10 or 20 min in vitro treatment of salivary glands of late 3rd instar larvae of *Drosophila melanogaster* with 1 mg/ml benzamide (BM) at 24° C results in the specific induction of the 93D puff and at the same time all other chromosomal RNA synthesis is severely repressed. Incorporation of ^{3}H-uridine in the nucleolus is not affected. In glands heat shocked (37° C) for 20 min in presence of BM, all the temperature shock (TS) puffs are induced but they incorporate ^{3}H-uridine to a lesser extent than in glands heat shocked in absence of BM. The 93D puff, which is highly induced by either of the treatments alone, is relatively less active in glands exposed to TS and BM simultaneously. When a 10 min BM treatment (at 24° C) precedes a 20 min TS or when the BM treatment follows TS, the ^{3}H-uridine incorporation on all TS puffs is relatively less and significantly, in both cases, the 87C puff is much less active (more so in TS followed by BM treated glands) than the 87A puff. Also, in both these treatments, the 93D puff does not show any additive effect of the BM and TS treatments. These observations are discussed in the light of possible role of the 93D puff in modulating the heat shock response.

Introduction

The heat shock induced gene activity in polytene cells of *Drosophila* has been studied intensively in recent years and the alterations in cellular transcription and translation patterns following the exposure of *Drosophila* cells to elevated temperatures are now known in some detail (Ashburner and Bonner, 1979). When *D. melanogaster* larvae or their excised salivary glands are subjected to a brief temperature shock (TS) at 37° C, nine specific puff sites are induced (Ritossa, 1964; Ashburner, 1970), and at the same time the previously active puffs are repressed in RNA synthesis and also the translation of previously existing mRNAs is strongly inhibited. In relation to the newly induced puffs, a characteristic set of heat-shock polypeptides appear in these cells (Tissieres

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et al., 1974; Spradling et al., 1975, 1977; Lewis et al., 1975; McKenzie et al., 1978; Ashburner and Bonner, 1979). In view of these and other observations, it has been suggested that gene expression in heat shocked cells of *Drosophila* is controlled at the level of transcription as well as translation (Mirault et al., 1978).

Mukherjee and Lakhotia (1979) have shown that of the nine puffs specifically induced by TS in *D. melanogaster* polytene nuclei the puff 93 D has an independent response to TS in contrast to the other TS puff sites which respond in a co-ordinated manner in a nucleus. Besides, the 93 D puff can also be selectively induced under certain conditions without concurrent induction of other TS puff loci (Lakhotia and Mukherjee, 1970; Bonner and Pardue, 1976) and apparently, the RNA synthesized at the 93 D locus in response to TS is not polyadenylated (Spradling et al., 1977). In view of these and some other unique features of the 93 D puff (Ashburner and Bonner, 1979), it appears to us that the 93 D puff may have a key role in modulating the heat shock response and we have directed our efforts to examine the changes in heat shock response following experimental alterations of activity of the puff at 93 D. In a preliminary report, Lakhotia and Mukherjee (1970) reported that a brief in vitro treatment of larval salivary glands of *D. melanogaster* with benzamide (BM) severely inhibits all chromosomal RNA synthesis but the activity of 93 D puff is stimulated several fold. Thus to examine the effects of changes in 93 D puff activity on the TS response, we have now studied the effects of BM and different combinations of heat shock and BM treatments on the transcriptional activity of the major TS puffs in polytene nuclei of *D. melanogaster*.

Material and Methods

A wild type strain (Oregon R) of *D. melanogaster* has been used for these studies. Eggs were collected for intervals of 1h and the larvae were grown on standard Agar-maize powder-brown sugar-yeast food at $24 \pm 1°$ C. Salivary glands from actively migrating late 3rd instar larvae were dissected out and exposed to BM or heat shock or to combinations of BM and heat shock in an incubation medium containing the inorganic salt constituents of Poels' (1972) tissue culture medium. The following treatments have been applied:

1. Benzamide (BM) Treatment. Benzamide (BDH, Poole) was freshly dissolved (1.0 mg/ml) in the incubation medium and the excised glands were treated with BM for 10 or 20 min at 24° C. In each case, the sister glands, kept as "controls", were incubated in BM-free medium for 10 or 20 min, respectively, at 24° C. The 10 or 20 min BM-treated glands were labelled with ^{3}H-uridine (500 μCi/ml; sp. act., 10.9 Ci/mM; BARC, Trombay) for 10 min in presence of BM. The control sister glands were also similarly labelled with ^{3}H-uridine in BM-free medium.

2. Heat Shock (TS) to Glands. Freshly excised salivary glands in salt medium were transferred to an incubator at $37 \pm 0.5°$ C and kept for 20 min. Following the TS, the glands were labelled with ^{3}H-uridine at 24° C for 10 min as above.

3. Combined BM and TS Treatments. Freshly excised glands were incubated at 37° C in the salt medium containing BM (1.0 mg/ml) for 20 min. The "control" sister glands were incubated in BM-containing medium at 24° C for 20 min. After the 20 min period, the "treated" (20 min BM treatment at 37° C) and "control" (20 min BM treatment at 24° C) glands were labelled with ^{3}H-uridine in presence of BM for 10 min as above.

4. BM Treatment Followed by TS. The "treated" glands in this set were first exposed to BM (1.0 mg/ml) for 10 min at 24° C following which they were transferred to BM-free medium at 37° C for 20 min. The "control" sister glands were given a 10 min BM treatment at 24° C and following this, were transferred to BM-free medium and incubated for 20 min at 24° C. The "control" and "treated" glands were then labelled with ^{3}H-uridine in BM-free medium at 24° C for 10 min as in other cases.

5. TS Followed by BM. The "treated" glands were first given a heat shock at 37° C in BM-free medium for 20 min and were then transferred to BM-containing medium (1.0 mg/ml) at 24° C for 10 min. The "control" sister glands were incubated in BM-free medium at 24° C for 20 min and were then treated with BM at 24° C for 10 min. The "control" and "treated" glands were finally labelled with ^{3}H-uridine in presence of BM for 10 min.

In each of the above experiment sets, at least 5 sister gland pairs have been used for the "control" and "treated" preparations and in each case, the glands were pulse labelled with ^{3}H-uridine for 10 min at 24° C with or without BM being present in the labelling medium as noted above for specific cases. The pulse labelled glands were briefly fixed in fresh aceto-methanol (1:3) and squashed in 50% acetic acid. After the removal of coverslips, the preparations were treated with 5% trichloroacetic acid at 4°–6° C for 10 min, thoroughly washed, dehydrated and coated with Ilford L_4 nuclear emulsion (1:1 dilution) for autoradiography. The coated slides were exposed in the dark at 4°–6° C for 4–5 days and were developed and fixed in the routine manner. The stained and mounted preparations were scored for the numbers of silver grains present on the five major TS puff sites (63BC, 87A, 87C, 93D, and 95D) in different well spread nuclei in sister "control" and "treated" glands. An attempt was made to score all the 5 puff sites in a nucleus. However, sometimes one or two puff sites had overlapping regions and in such instances the other clearly identifiable puff sites only were scored. As mentioned later in Observations, in the case of BM treatment experiments, preparations from the "control" and "treated" glands were also scored for the degree of labelling of certain other regions to assess the general effects of BM treatment on nuclear transcription.

Observations

1. Effect of Benzamide on RNA Synthesis Patterns

Earlier reports (Lakhotia and Mukherjee, 1970; Lakhotia, 1971) had indicated that a 10 min in vitro treatment of larval salivary glands of *D. melanogaster* with BM causes a several-fold increase in ^{3}H-uridine incorporation at the 93D puff while RNA synthesis at all other chromosomal loci is significantly inhibited. The present detailed observations on the effects of 10 and 20 min BM treatments confirm the preliminary report. Data on the ^{3}H-uridine labelling of 93D puff and certain other chromosomal regions in 10 and 20 min treated and respective control preparations are presented in Table 1. In each of the 10 or 20 min BM treated glands, the general chromosomal labelling is inhibited by a factor of 2 or more as reflected in the mean grain counts on chromosome segments 1A to 3C on the X-chromosome and 61A to 63C on 3L in BM treated and sister control glands (see Table 1 and Fig. 1). The ^{3}H-uridine incorporation by the β-heterochromatin region of chromocentre also declines after BM treatment in paraellel with the chromosomal labelling (See Table 1). The nucleolar uptake of ^{3}H-uridine is not much affected by 10 or 20 min BM treatment since the mean grain density (mean no. of grains on 25 μm^2 area) of the nucleolus in different nuclei in control and treated glands remains nearly similar (Table 1). In contrast, the 93D puff shows a several-fold increase in ^{3}H-uridine uptake

Table 1. Effect of in vitro 1 mg/ml benzamide (BM) treatment on ^{3}H-uridine incorporation in polytene nuclei of *D. melanogaster*

Treatment[a]		Mean (±S.E.) grain density				
		93D	X segment (1A–3C)	3L segment (61A–63C)	Chromo-centre	Nucle-olus[b]
1. 10 min BM	Control	22.5±1.3 (38)[c]	98.5±6.6 (37)	120.0±5.2 (37)	41.0±1.0 (29)	34.6±1.7 (35)
	Treated	87.4±4.7 (56)	52.9±1.2 (45)	52.9±1.2 (45)	28.6±1.4 (42)	30.9±1.0 (48)
2. 20 min BM	Control	20.5±2.0 (34)	80.0±6.1 (32)	75.7±6.3 (32)	21.3±2.4 (22)	27.7±0.5 (35)
	Treated	60.0±3.2 (42)	27.2±1.3 (33)	31.1±2.0 (34)	6.6±0.2 (32)	30.0±0.7 (35)

[a] The data for "control" and "treated" in each treatment are pooled from 5 pairs of sister glands

[b] For nucleolar grain density, grains were counted on three separate regions (each 25 μm^2) of nucleolus in a nucleus

[c] Figures in parentheses indicate the total number of nuclei examined in each case

in 10 min BM treated glands compared to that in sister control glands (Table 1, Fig. 1). Concurrently, the size of the 93D puff also increases after BM treatment. A 20 min BM treatment causes, on an average, a greater repression of chromosomal RNA synthesis while the 93D puff activity remains induced (Fig. 1c), although the extent of ^{3}H-uridine uptake at 93D puff in 20 min BM treated glands is somewhat less than in 10 min BM treated glands (see Table 1). It may also be noted here that in some rare glands in 10 and 20 min BM treated series, the 93D puff was not seen to be activated and in a few cases the degree of activation was even lesser (data not presented). Presumably, such variations in some glands may be due either to experimental or to developmental conditions of glands. Also the degree of repression of the general chromosomal RNA synthesis varied in different glands from near total to about 50%.

The pre-existing puffs do not regress after BM treatment even though the ^{3}H-uridine incorporation in them is severely repressed. In general however, it appears that the ^{3}H-uridine incorporation at many of the very active puff sites is relatively less sensitive to the BM treatment than that at smaller puffs and non puffed regions.

None of the TS puffs, other than 93D, are stimulated by the 10 min BM treatment as can be seen from the data in Table 2. The mean grain counts on the 4 other major TC puff loci are in fact much less in BM-treated preparations than in corresponding "control" preparations. A similar situation has been noted for 20 min treated glands (detailed data not presented).

BM treated glands recover very quickly since if BM is not present during the ^{3}H-uridine labelling period, the 93D labelling is nearly similar to or only marginally more than that in control and also the inhibitory effect on general chromosomal RNA synthesis is much less marked. The detailed data on this aspect are not presented but an indication of the recovery may be had from

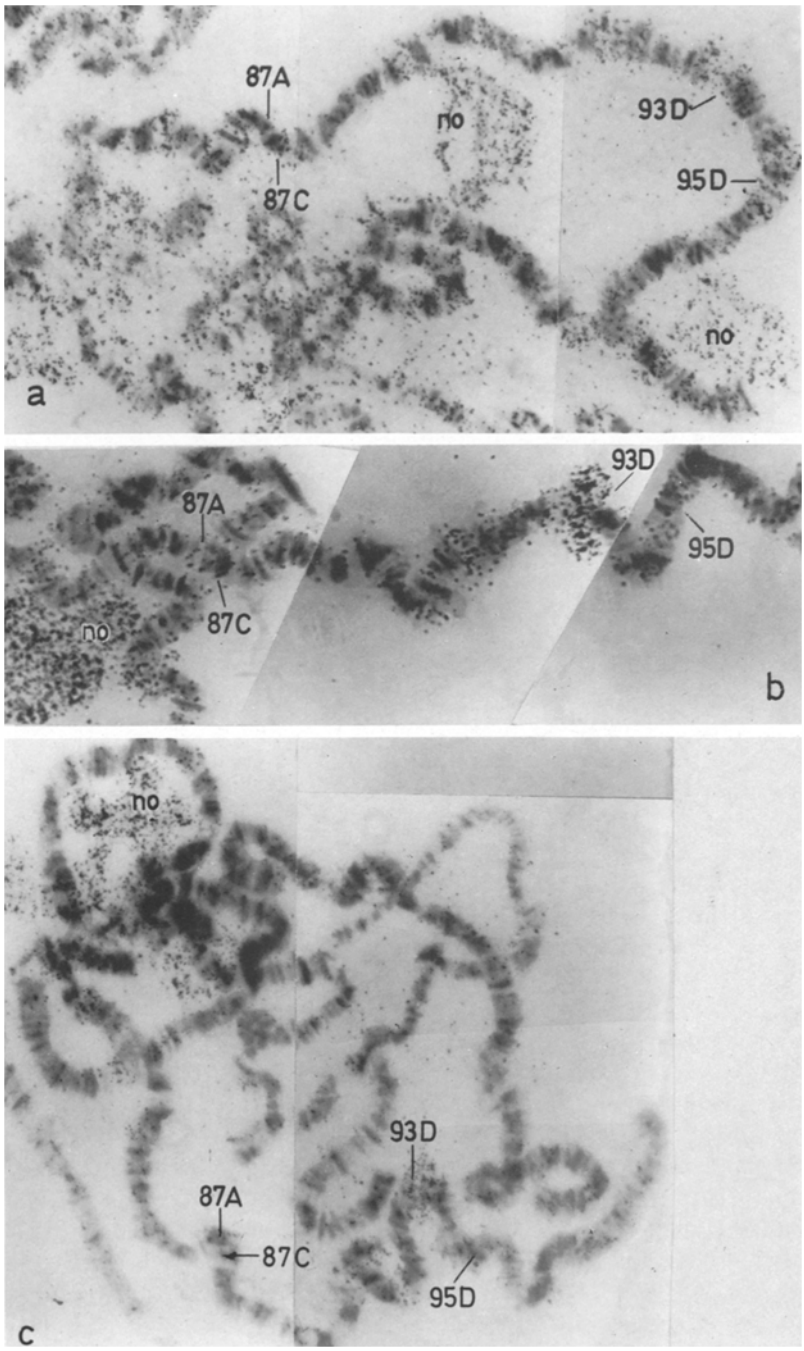

Fig. 1 a–c. Autoradiographs of ^{3}H-uridine labelled polytene nuclei from untreated control (**a**), 10 min BM treated (**b**) or 20 min BM treated (**c**) salivary glands. Note the low labelling of unpuffed 93 D region in control and the heavy labelling of the large puff induced at 93 D after 10 and 20 min BM treatment; while the overall chromosomal labelling is considerably less (more so in **c**) in BM treated nuclei compared to the control, the labelling of nucleolar region (*no*) is not different. Magnification in this and in Figure 2 is ×1,080

the grain densities on different TS puff sites in "control" preparations in the BM followed by TS experiment (Exp. 4 in Table 2). These glands were kept for 20 min in BM-free medium after the initial 10 min BM treatment at 24° C. As can be seen from Table 2, the labelling of all sites in these glands is similar to that in normal glands (Compare with the "control" in Exp. 1 in Table 2).

2. Effect of BM on Heat Shock Response

Examination of the autoradiographic preparations of glands exposed to BM or TS or to different combinations of BM and TS treatments, reveals very specific alterations in the transcription patterns of the major TS puff loci (see Fig. 2 and Tables 2 and 3). The data on the mean grain counts on the 5 TS puff loci in "control" and "treated" preparations of different experiment sets are presented in Table 2 to compare the degree of activation of individual puff sites under various conditions. The activity of each of the major TS puff locus relative to the 87A in each scored nucleus has also been measured in terms of ratio of grains on 87A and a given TS puff and the data on mean ratios are presented in Table 3 to reveal differential response of the five TS puffs to various combinations of BM and TS treatments.

As noted above, a 10 min BM treatment (at 24° C) stimulates only the 93D activity while the other TS puffs are not at all induced. As has been documented earlier, a routine heat shock to salivary glands of *D. melanogaster* induces 9 specific puff loci and of these, some puffs are more active than others (Ashburner, 1970; Mukherjee and Lakhotia, 1979). Further, as the latter authors have shown, the highest mean grain count is over the 93D puff (Table 2) although in individual nuclei, its labelling density varies independent of the other TS puffs. The 87A and 87C puffs are much more heavily labelled than 63BC and 95D, and in a given nucleus the activity levels of the two puffs are nearly equal (mean 87A/87C ratio = 1.0, see Table 3 and Fig. 2a).

In glands treated with BM at 37° C for 20 min (Exp. 3, Table 2) the overall ^{3}H-uridine incorporation on different TS puffs is less than in glands given a heat shock in BM free medium (see Fig. 2b and Table 2), but interestingly, while the mean grain counts on 87A and 87C puffs are more affected, the grain counts on 63BC and 95D puffs are only marginally less than in routine heat shocked glands. Furthermore, while BM alone induces the 93D puff maximally, the two treatments combined together cause a lesser induction of 93D than either of the treatments given individually (see Table 2). A comparison of the activity of 63BC, 87C, 93D, and 95D relative to 87A in glands simultaneously exposed to BM and TS, reveals that while the 87A/87C ratio remains close to one, as in normal heat shocked glands, the 87A/63BC and 87A/95D ratios are significantly decreased compared to those in only heat shocked glands (compare rows 1 and 2 in Table 3) and the 87A/93D ratio is slightly (although not significantly, $P > 0.05$) increased in BM + TS treated glands.

In glands first exposed to BM for 10 min at 24° C and then to TS in BM free medium for 20 min (BM followed by TS, Exp. 4 in Table 2, Fig. 2c–d), the mean grain counts on all TS puffs are much less than in only heat shocked

Table 2. ^{3}H-uridine incorporation on the 5 major heat shock puff loci after benzamide (BM), temperature shock (TS) or different BM and TS combinations

Experiment	Treatment[a]	Mean (±S.E.) no. of silver grains on major TS puff loci[b]				
		63BC	87A	87C	93D	95D
1. BM	"Control" (BM-,24° C, 10 min)	11.59±0.7 (37)	5.89±0.7 (19)	5.11±0.6 (19)	22.54±1.3 (38)	8.18±0.6 (37)
	"Treated" (BM+, 24° C, 10 min)	1.08±0.02 (54)	1.12±0.03 (35)	1.07±0.02 (35)	87.38±4.68 (38)	1.03±0.02 (49)
2. TS	"Treated" (BM)−, 37° C, 20 min)	40.09±1.7 (41)	65.32±2.5 (38)	64.98±3.1 (38)	76.12±2.5 (42)	22.72±3.8 (39)
3. TS+BM	"control" (BM+, 24° C, 20 min)	5.65±0.7 (37)	1.28±0.02 (36)	1.31±0.3 (36)	35.97±2.0 (38)	4.81±0.4 (38)
	"Treated" (BM+, 37° C, 20 min)	37.57±2.5 (56)	38.96±2.8 (57)	44.28±3.0 (57)	32.79±2.8 (57)	19.65±1.6 (57)
4. BM followed by TS	"Control" (BM+, 24° C, 10 min→ BM−, 24° C, 20 min)	7.45±0.6 (31)	3.68±0.5 (31)	3.16±0.4 (31)	22.03±1.2 (34)	8.86±0.6 (35)
	"Treated" (BM+, 24° C, 10 min→ BM−, 37° C, 20 min)	16.54±0.9 (39)	33.84±1.5 (38)	18.68±0.4 (38)	35.25±2.7 (39)	10.03±0.8 (36)
5. TS followed by BM	"Control" (BM-, 24° C, 20 min→ BM+, 24° C, 10 min)	5.68±0.6 (35)	1.92±0.3 (36)	1.56±0.3 (36)	40.49±1.6 (39)	6.37±0.2 (37)
	"Treated" (BM-, 37° C, 20 min→ BM+, 24° C, 10 min)	19.67±1.0 (37)	43.10±2.1 (41)	9.36±0.9 (41)	33.72±2.0 (40)	14.29±0.6 (38)

[a] The entries in parentheses in this column sequentially indicate if the glands were incubated in presence (BM+) or absence (BM-) of benzamide, the incubation temperature (24° C or 37° C) and the duration (10 or 20 min) of treatment; the arrows in the last four treatments indicate the subsequent treatment schedule before the glands were labelled with ^{3}H-uridine; see Materials and Methods for details; the "control" and "treated" glands in each experiment are sister glands

[b] The numbers in parentheses indicate the number of nuclei examined for each of the puff site

glands (Exp. 2 in Table 2). In this case, although the 93D puff shows maximum mean grain density among the TS puff loci in "treated" glands, this grain density is reduced to nearly half that observed in only heat shocked or only BM treated glands. Significantly, the 87C puff in glands exposed sequentially to BM and TS is much less active than the 87A puff (Fig. 2c–d); thus the 87A/87C grain ratio in these glands is close to 2.0 rather than 1.0 as in normal heat shocked glands (Table 3). On the other hand, the relative activity of 93D and 95D puff loci in these glands is similar to that seen after normal heat shock (Table 3). The 63BC puff also appears to be less active as compared

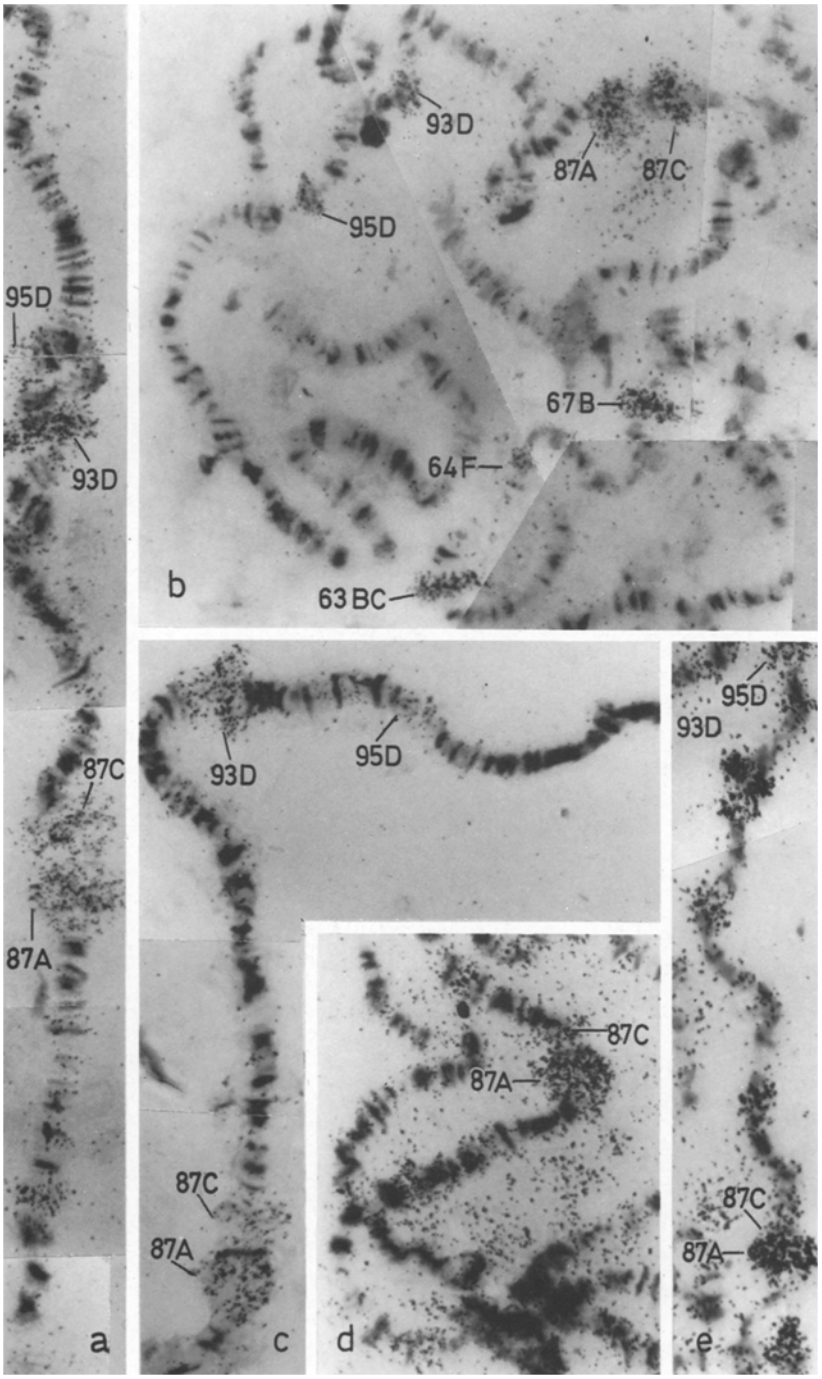

Fig. 2a–e. Parts of autoradiographs of the ^{3}H-uridine labelled polytene chromosomes to show the patterns of ^{3}H-uridine incorporation in TS puff sites in glands given 20 min TS (**a**), combined BM and TS (**b**), BM followed by TS (**c, d**) or TS followed by BM treatment (**e**). Note the inhibition of 87C puff in **c** to **e**. When BM precedes TS treatment, the 87C puff sometimes is developed as much as 87A (**c**) or is only marginally developed (**d**) but the ^{3}H-uridine labelling is always less than in 87A

Table 3. Relative ^{3}H-uridine incorporation on the major TS puff sites after TS or different TS and BM combination treatments

Treatment	Mean (±S.E.) ratios of grain densities			
	87A/87C	87A/93D	87A/95D	87A/63BC
20 min TS	1.0±0.03 (38)	0.9±0.1 (38)	3.1±0.2 (35)	1.7±0.1 (37)
BM+TS	0.9±0.04 (50)	1.4±0.1 (56)	2.1±0.1[a] (50)	1.1±0.3[a] (55)
BM followed by TS	1.9±0.1[a] (38)	1.1±0.1 (37)	3.4±0.7 (34)	2.3±0.2[a] (37)
TS followed by BM	6.4±0.8[a] (41)	1.3±0.1 (38)	3.1±0.1 (38)	2.4±0.1[a] (37)

[a] Ratios are significantly different at $P<0.05$ from the corresponding ratios in 20 min heat shocked glands

to the 87A puff, since 87A/63BC grain ratio shows significant increase over that in only heat shocked glands.

Glands first exposed to heat shock and then to BM at 24° C (Exp. 5, Table 2, Fig. 2c) again show a response qualitatively comparable to that of the "treated" glands in the previous experiment (BM followed by TS, Exp. 4; Table 2). However, in these glands, the 87C puff shows very little induction both in its size and in ^{3}H-uridine incorporation. Thus the mean 87A/87C grain ratio in this experiment is 6.37 as compared to 1.0 in only heat shocked glands (see Table 3). The 63BC puff too is relatively less active in these glands since the 87A/63BC grain ratio is significantly higher than in normally heat shocked glands (Table 3).

Discussion

The response of the 93D puff locus to heat shock is unique (Mukherjee and Lakhotia, 1979; Ashburner and Bonner, 1979). In this context the action of BM in specifically inducing only the 93D puff is very interesting. The precise mode of action of BM in causing the observed effects on transcription patterns in polytene nuclei is not known. Earlier report of Sirlin and Jacob (1964) had shown that in polytene nuclei of *Smittia*, in vitro BM treatment severely inhibits all chromosomal RNA synthesis without affecting the nucleolar transcription. In this respect, the present results agree with the earlier findings in *Smittia* since in *Drosophila melanogaster* polytene nuclei also the BM treatment inhibits general chromosomal RNA synthesis while the nucleolar uptake of ^{3}H-uridine remains unaffected. But the most ineresting aspect of BM effect in *Drosophila* polytene nuclei is the induction of a specific puff site. It may be noted here that in several other species of *Drosophila*, viz. *D. ananassae, D. kikkawai, D. hydei, D. nasuta* etc., also we have observed a similar BM induced activation of a single puff site and interestingly, in all these species,

the BM induced puff is also induced by the TS (unpublished observations). Thus it seems that the in vitro BM treatment causes some specific alterations in polytene cell physiology which in turn induce the specific activation of 93D puff in *D. melanogaster* while the other loci are repressed. There are certain common features in the response of polytene nuclei to heat shock and to BM. Both these treatments inhibit general chromosomal RNA synthesis without much initial effect on ^{3}H-uridine incorporation in nucleolus. The TS and BM treatments also induce specific puff sites, but while TS induces a set of loci, BM activates only one of the TS puff loci. At present we do not know if like TS, the BM treatment also affects the translational machinery of polytene cells; we are presently analysing this aspect. Nevertheless, in view of the above similarities in their effect on nuclar transcription patterns, it seems that of the several levels of cellular physiology at which TS acts (Mirault et al., 1978), the BM may share some so that the response to these two treatments is similar in certain respects. The possibility of a partially common target is further strengthened by our observations on the response of different TS puff loci to the various combinations of BM and heat shock treatments.

The response of 93D puff to different BM and TS combination treatments is very interesting. While individually, either of the treatments induce 93D puff maximally, their combinations appear to restrict the 93D activity. In glands exposed to either TS and BM simultaneously or to TS followed by BM, the 93D activity is induced to a lower level than in their respective BM-treated "control" sister glands or than the 87A puff in the same glands. These observations show that one treatment alters the target for the other so that the total response is modified. Thus in TS followed by BM treated glands the initial heat shock would have induced the 93D puff maximally (on average), but the subsequent BM treatment not only does not further induce 93D, but actually causes this and other TS puffs to become less active. Thus in combination experiments, the response to both treatments gets altered. However, the mechanism by which they interact is not clear at present. Nevertheless, in view of the specific inducibility of the 93D puff by BM, it appears to us that the modifications in TS response in conjunction with BM treatment may be due to some role of the 93D puff activity. In the treated glands exposed first to BM and then to TS, the 93D puff is more active than in their "control" (chased in BM free medium after initial BM treatment) sister glands or than the 87A puff in the same nuclei. This aspect may be related to the rapid recovery of glands from BM effect after the drug is withdrawn so that in these treated glands, the response of the different puffs to subsequent TS is qualitatively similar to that after the normal heat shock, although quantitatively, each of the TS puff is still less active.

The considerably reduced activity of 87C puff in glands exposed to BM before or after the TS is very significant. Normally, the 87A and 87C puffs are nearly equally active in heat shocked glands as seen in their size and their ^{3}H-uridine incorporation. It is also known that these two puff sites are duplicated gene loci, both coding for the same 70,000 dalton heat shock polypeptide (Livak et al., 1978; Schedl et al., 1978; Ish-Horowicz et al., 1979). These two loci,

however, do differ in some of the DNA base sequences and it has been speculated (Ish-Horowicz et al., 1979) that these two duplicated loci may be under different regulatory controls. Our present observations that the 87C puff, when BM treatment precedes or follows TS, is much less active than the 87A puff shows that the activity of these two duplicated loci can indeed be independently regulated under experimental conditions and this may have implication for their in vivo regulation also. We have seen that when TS is given in BM free medium to glands previously exposed to BM at 24° C, the activity of 87C is reduced relative to 87A by a factor of 2 while in glands exposed to BM after the TS, the 87C puff shows very little activity and ^{3}H-uridine incorporation on this locus is about 6–7 times less than on 87A. In the first case, it appears that since the glands recover rapidly from the BM effect, the 87C puff is affected to a lesser degree than when the BM treatment is given after TS. It is interesting, however, that when the BM treatment is concurrent with TS, the 87A and 87C puffs are equally less active in response to TS. A further molecular analysis of alterations in transcriptional and translational patterns under these different treatment conditions would provide information about the independent regulation of the duplicated loci at 87A and 87C. The characteristic changes in the relative activity of 63BC puff after BM and TS combination treatments also warrant further analysis.

It may also be noted here that the specific interaction of TS and BM treatments may vary depending upon the composition of the medium in which the glands are incubated. We have observed (Lakhotia and Mukherjee, 1979; also see Mukherjee and Lakhotia, 1979) that when these same combination treatments are given in a *Drosophila* Ringer solution (Berendes et al., 1965), the chromosomal RNA synthesis in all cases is totally inhibited and in glands exposed to TS in presence of BM in the Ringer solution none of the TS puffs are induced. In the present case, where the glands are incubated in a more complex salt medium, modified after Poels (1972), the BM and TS combination treatments give different results. Thus it seems that the action of puff inducing agents can be modified by the constituents of the incubation medium as has already been noted by Bonner and Pardue (1976) that heat shock in aged Graces' medium induced only the 93D puff.

The present observations thus show that the TS response may be modulated in a characteristic manner by benzamide treatment which by itself activates only the 93D puff. This suggests that the 93D puff may be involved, in some manner, in regulating some of the heat shock induced perturbations in cellular transcription and translation patterns. Also, the possibility of specifically inducing, by BM treatment, extensive RNA synthesis at 93D when other loci are significantly repressed, would permit easy isolation and characterization of RNA made at 93D and this in turn, would facilitate an understanding of this unique TS puff.

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